Experimental Cancer Cachexia Induced by Transplantable Colon 26 Adenocarcinoma in Mice

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ABSTRACT

The present study investigates a tumor model for cachectic mice. Among various murine transplantable tumors, used for assessing cytostatics, we identified colon 26 adenocarcinoma (colon 26) as capable of causing cachexia. Fifteen days after inoculation, the tumor grew to about 6% of the body weight causing substantial carcass weight loss of 3.4 g (14.5% of the carcass weight). When the tumor size was 2.7 g at 3 weeks after the inoculation, the carcass weight was 12 g less than the age-matched control. The tumor continued to grow while the mice maintained this weight, surviving for an average of 45 days. This extensive weight loss was essentially the wasting of adipose and muscle tissues. Hypoglycemia, and hypercorticism, although some mild disorders of hepatic functions were observed. Additions, the colon 26 caused disorders of hepatic functions: the concentration of acute phase proteins in serum increased; the number of hepatic cytosol receptors decreased; and activities of hepatic catalase and drug-metabolizing enzymes decreased. On the other hand, noncachectic mice with Meth A fibrosarcoma gained weight, which was somewhat less than the control, and had neither hypoglycemia nor hypercorticism, although some mild disorders of hepatic functions were found. Mice bearing colon 26 is an appropriate model for elucidating the mechanism that causes cachexia.

INTRODUCTION

The cachexia, which includes weight loss, anorexia, asthenia, and anemia, resulting from tumor growth complicates therapeutic intervention (1-3). Even before disseminated cancer is diagnosed, almost half of the patients are reported to have lost some weight (2), and the prognosis of cancer patients with cachexia is poor (3). Prechemotherapy wasting is associated with a shorter survival time for most tumor types (2) and with a generally lower response rate to chemotherapy (3, 4). Therefore, an appropriate treatment modality is necessary to counteract cachexia.

Antitumor agents have generally been assessed by using mice bearing small burdens of transplantable tumors, particularly mice that had just been implanted with tumor cell suspension, which induce only minor disorders of host heomeostasis. Therefore, this model does not completely reflect the condition of patients on chemotherapy, who have many disorders of homeostasis (1). In mice, even after most of transplantable tumors have grown to an appreciable size, there is neither weight loss nor tissue wasting (5). Only few animal transplantable tumors and human tumor xenografts are able to induce cachexia and disorders of homeostasis (5-11).

We have examined various murine transplantable tumor lines, used for assessing cytostatics, for their ability to induce cachexia while tumor burdens are still relatively small. These studies identified murine colon 26, an undifferentiated carcinoind induced by the carcinogen N-nitroso-N-methylurethan (12), as capable of causing cachexia. In this paper we describe physiologic changes associated with tumor cachexia in several mouse tumor models and show that the colon 26-bearing mice are indeed cachectic.

MATERIALS AND METHODS

Mice. Male BALB/c × DBA/2 F1 (hereafter called CD2F1) and C57BL/6 × C3H/HeN F1 (hereafter called B6C3F1) (SLC, Hamamatsu, Japan) mice were used at the age of 5 weeks. They were fed breeding diet F1 (Funabashi Farm, Funabashi, Japan), containing 21.3% protein, 57.1% carbohydrates, 5.6% fat, 3.3% fiber, 5.7% ash, and 7.0% moisture (metabolic calories, 3.76 kcal/g), and water ad libitum.

Tumors. Murine colon 26 adenocarcinoma cells were cultured in vitro with RPMI 1640 containing 10% fetal calf serum. By treating the cells with trypsin, we obtained a single cell suspension, and 106 cells were then inoculated s.c. into the inguinal flank of CD2F1 mice. Other transplantable tumors described in Table 1 were passaged in vitro in culture or in vivo and inoculated s.c. into F1 mice (0.2-2 × 106 cells). UV2237m fibrosarcoma and K1735m melanoma were kindly supplied by Dr. I. J. Fidler, M. D. Anderson Hospital and Tumor Institute, Houston, TX.

Measurement of Body Wasting and Food Intake. Body weight and tumor size (length and width) were measured 2 or 3 times a week. Carcass weight, the difference in weight between the whole body and tumor tissue, was measured. The tumor weight (g) was sometimes estimated by using the equation

\[a^2/b \times F\]

where a, b, and F are length and width (cm) of tumor and a correction factor, respectively. The correction factor was determined by comparing the actual tumor weight (g) with tumor volume (a^2/b × F cm^3) in separate experiments. Weight changes in the left epididymal adipose tissues and gastrocnemius muscles of the left hindleg were also measured. The amount of food consumed by one mouse per cage was calculated from the weight of the feed that remained by 12 a.m. each day.

Determination of Body Composition. Body composition was determined as described elsewhere (7). Mice bearing colon 26 and the age-matched control mice were killed, and the tumors in the respective mice were dissected out. The animals and tumor tissues were freeze-dried under reduced pressure, and their dry weights were measured. The lipids were then extracted from the carcass first with chloroform: methanol (1:1), then with ethanolaacetone (1:1), and finally with diethyl ether. The extracts were pooled and dried, and the amount of lipids was quantified by weighing with an accuracy of ±1 mg. The weight of lean mass was calculated as the difference between carcass and total lipid dry weights.

Assays. Blood samples were collected from the heart of chloroform-anesthetized mice via puncture for most assays and from the orbital veins of nonanesthetized mice by retroorbital bleeding with a capillary pipet for the corticosterone assay and peripheral blood cell count. The samples for the corticosterone assay were collected between 9 and 10 a.m. to minimize hormone fluctuations based on the circadian rhythm. Peripheral blood samples were stained with Türk's solution and the number of nucleated cells was counted. For the differential cell counts, peripheral blood smears were fixed with methanol and stained with Diff Quick stain (Kokusai Shiyaku, Kobe, Japan). For determination of the blood concentration of various substances to be tested, the

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1 To whom requests for reprints should be addressed.
Table 1 Carcass weight changes in mice bearing various transplantable tumors

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Carcass wt changes (g) at following tumor wt</th>
<th>No. of tumor cells inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.0 g</td>
<td>4.0 g</td>
</tr>
<tr>
<td>In CD2F, mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon 26</td>
<td>-0.8 (17)</td>
<td>-3.9 (28)</td>
</tr>
<tr>
<td>Meth A</td>
<td>1.4 (12)</td>
<td>1.8 (15)</td>
</tr>
<tr>
<td>S180</td>
<td>1.8 (14)</td>
<td>1.4 (19)</td>
</tr>
<tr>
<td>Ehrlich</td>
<td>2.3 (14)</td>
<td>1.4 (17)</td>
</tr>
<tr>
<td>In B6C3F, mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV 2237m</td>
<td>2.5 (18)</td>
<td>1.4 (22)</td>
</tr>
<tr>
<td>K 1735m</td>
<td>3.3 (18)</td>
<td>3.2 (21)</td>
</tr>
<tr>
<td>B16F1</td>
<td>2.0 (16)</td>
<td>0.9 (20)</td>
</tr>
<tr>
<td>LLC</td>
<td>1.6 (15)</td>
<td>0.3 (18)</td>
</tr>
<tr>
<td>A755</td>
<td>1.8 (8)</td>
<td>1.4 (10)</td>
</tr>
<tr>
<td>Colon 38</td>
<td>2.9 (40)</td>
<td>1.3 (52)</td>
</tr>
</tbody>
</table>

* A small piece of tumor tissue (1 mm³) was inoculated s.c. into a mouse with a trocar.

following methods and reagents were used: an o-toluidine color reaction method for measuring serum glucose (13); a clotting time test with thrombin for plasma fibrinogen (14); an immunodiffusion assay with anti-mouse IAP antibody (Saikin Kagaku Institute, Sendai, Japan) for plasma IAP (15); a Kyowa Medix assay kit (Tokyo, Japan) for sialic acid contents of plasma proteins (16); and a radioimmunoassay method with rabbit anti-corticosterone antibody (Paesel GmbH & Co., Frankfurt, West Germany) and [3H]corticosterone (New England Nuclear, Boston, MA) for serum corticosterone (17).

Liver extracts were homogenized with phosphate-buffered saline solution (pH 7.4), and its catalase activity and receptor capacity for binding to glucocorticoid hormones were determined by an H₂O₂ consumption assay (18) and by a binding assay method with [3H]dexamethasone (Amersham International, Amersham, Buckinghamshire, England) (19), respectively. The amount of P-450 enzymes in the homogenate was determined by a spectrophotometric assay as described by Omura and Sato (20).

Length of Sleep Induced by Pentobarbital. The ability of the mice to eliminate pentobarbital (40 mg/kg i.p.) was indirectly measured by comparing the length of sleep of non-tumor-bearing and tumor-bearing mice (21).

Statistics. Differences in tumor size, tissue weight, enzyme activities, and concentrations of substances were compared by using the Mann-Whitney U test. Differences were considered to be significant when the probability value was less than 0.05 (P < 0.05).

RESULTS

Weight Loss Induced by Tumors. Various murine transplantable tumors were examined for their ability to induce tumor cachexia, based on how much carcass weight had been lost during tumor growth (Table 1). The carcass weight loss of mice bearing colon 26 was 3.9 g when the tumor size reached 4 g, and the loss is significant if compared with the weight gain of the age-matched control (about 5 g). In other tumors the carcass weight change was only slightly at a degree ranging from 0.8 to 1.7 g even when the tumor size reached 6 g. In addition, piloerection and asthenia were observed only in mice bearing colon 26.

Food Intake, Weight Loss and Tissue Wasting in Colon 26-bearing Mice. Between days 8 and 28, the cumulative food intake of the colon 26-bearing mice [81.03 ± 9.45 (SD) g/mouse, n = 6] was similar to that of the age-matched control [81.57 ± 4.11 (g/mouse, n = 6]. On the other hand, the tumor-bearing mice lost a significant amount of carcass weight beginning on day 15, when the tumor was 1.6 g (6.2% of the body weight) (Fig. 1), and they continued to lose weight until they weighed 13 g less than the age-matched control on day 30. The mice maintained this weight while the tumor continued to grow. In total, the mice survived for an average of 45 days. In a separate experiment, after the primary tumor was dissected out on day 24, the mice gained 7.3 ± 1.2 g, and piloerection and asthenia were improved within 5 days.

The loss of carcass weight during tumor growth was essentially the wasting of muscle and adipose tissues. The wasting of epididymal adipose tissue and gastrocnemius muscle was observed 2–3 weeks after the tumor inoculation (Fig. 2). After 3 weeks, the adipose tissue was almost entirely depleted. Histological analysis of the body components further confirmed that this substantial tissue wasting was due to the loss of total body fat and lean body mass but not that of water content (Table 2).

Abnormalities in Colon 26-bearing Mice. Physiological changes associated with cachexia were monitored during the process of the colon 26-induced wasting. Hypoglycemia began around day 14 (Fig. 3) when the body weight was not so obvious (Fig. 1) and maintained even after the tumor weight had increased 3-fold. Serum concentration of glucocorticoid hormone was significantly increased during the drastic reduction in carcass weight beginning approximately on day 18,
Although its deviation among mice was large (Fig. 4). The content of hepatic cytosol receptors for glucocorticoid hormones decreased to 39.2 fmol/mg protein on day 22 compared with 99.1 fmol/mg protein in the age-matched control. Other abnormalities in mice bearing colon 26 included a longer sleep induced by pentobarbital, which was administered for predicting the capacity of the mice to metabolize drug, and a reduction in hepatic P-450 drug-metabolizing enzyme activity (Table 3). On day 29 after the tumor inoculation, the length of sleep was >50 times longer, and P-450 enzyme activity was reduced to one-fourth, compared with the non-tumor-bearing control.

Catalase activity in the liver was slightly reduced on day 21 although its deviation among mice was large (Fig. 4). The content of hepatic cytosol receptors for glucocorticoid hormones decreased to 39.2 fmol/mg protein on day 22 compared with 99.1 fmol/mg protein in the age-matched control. Other abnormalities in mice bearing colon 26 included a longer sleep induced by pentobarbital, which was administered for predicting the capacity of the mice to metabolize drug, and a reduction in hepatic P-450 drug-metabolizing enzyme activity (Table 3). On day 29 after the tumor inoculation, the length of sleep was >50 times longer, and P-450 enzyme activity was reduced to one-fourth, compared with the non-tumor-bearing control.

Catalase activity in the liver was slightly reduced on day 21 when the body weight wasting was severe but was significantly low on day 29 (Table 3). On the other hand, the concentrations of acute phase proteins in the blood, such as fibrinogen, IAP, and sialic acid-rich glycoproteins, were elevated and approached maximum values on day 14 when the body wasting was only slight (Table 4). The hematological tests of the mice bearing colon 26 showed that severe leukocytosis occurred (Fig. 5). During tumor growth, the number of leukocytes began to increase approximately on day 14 after the tumor inoculation, until the number was greatest (11-fold) on day 33. We attribute the leukocytosis to a change in the proportion of neutrophils, which increased from 17.3 ± 4.2% (n = 3) of the control to 74.0 ± 16.3% (n = 3) of colon 26-bearing mice on day 22. On the other hand, the number of RBC decreased by less than 10% (data were not shown).

Physiological Changes in Mice Bearing Meth A. Table 5 shows physiological changes in CD2F mice bearing either colon 26 carcinoma or Meth A fibrosarcoma. Mice bearing Meth A did not cause weight loss even when the tumor size reached one-fourth of the whole body weight. Hypoglycemia and hypercorticism were evident only in colon 26-bearing mice. Other changes such as leukocytosis, an increase in the concentration of acute phase protein, and hepatic malfunctions were observed in these two tumor models, although the degree was much larger in mice bearing colon 26 than in those bearing Meth A.

**DISCUSSION**

We have examined murine tumors and found that mice bearing colon 26 carcinoma caused an extensive carcass weight loss of up to 41% of the body weight of the age-matched control and also had hypoglycemia and hypercorticism. Mice bearing other tumors had minor changes in the carcass weight while the tumor grew up to 4 or 6 g. Hypoglycemia and hypercorticism were not observed in mice with Meth A fibrosarcoma. These physiological changes have also been reported for known cachectic models: colon adenocarcinoma MAC16 (11); MCA fibrosarcoma (22, 23); and sarcoma MCG101 (7, 24). Thus, our results suggest that colon 26 induces cachexia.

Some possible mechanisms can be considered for the wasting in cachexia. It may result from lack of nutrient intake because of either anorexia or intestinal malfunction. It has been reported that weight loss is associated with anorexia in some tumor-cachexia models (7, 22, 25, 26) but not in other cachexia models (11). It is unlikely that the cachexia in mice bearing colon 26 was due to anorexia, because food intake was not significantly reduced while they were losing weight. The weight loss could also have resulted from a metastasizing tumor. Since the substantial weight loss was reversed immediately after the primary tumor was dissected out, it is unlikely that the colon 26 tumor metastasized to particular sites in the body causes cachexia. The weight loss was accompanied by various physiological changes, some occurring before but others occurring after the substantial weight loss. Some of the changes may have triggered cachexia while others may have counteracted them. Although the decrease in the carcass weight was related to the tumor size until the third week of tumor inoculation, the carcass weight...
CANCER CACHEXIA INDUCED BY COLON 26 IN MICE

Fig. 3. Blood glucose concentration in mice bearing colon 26. Plasma samples were collected from colon 26 tumor-bearing (•) or non-tumor-bearing (○) mice (5 mice/group) and glucose concentration in the plasma was determined as described in “Materials and Methods.” *, P < 0.05 for the control group. Bars, SD.

Fig. 4. Concentration of corticosterone in serum of mice bearing colon 26. Serum samples were collected from mice bearing colon 26 (■) or non-tumor-bearing mice (○) (5 mice/group) and the concentration of corticosterone was determined as described in “Materials and Methods.” *, P < 0.05 for the control group. Bars, SD.

maintained constant despite 3-fold increase in tumor weight (Fig. 1). This may have resulted due to the alterations of energy metabolism which counteract the wasting.

Hypercorticism may induce cachexia in mice bearing colon 26. The increase in glucocorticoid hormone can explain some of the metabolic alterations that occur during cancer cachexia, such as the induction of hepatic enzymes promoting gluconeogenesis (27, 28), the enhancement of the synthesis of many acute phase proteins (29, 30), and the regulation of the production of possible mediators of cachexia, IL-1 (31) and TNF (32). Colon 26 caused hypercorticism during the process of the wasting; however, the changes in the serum concentration of this hormone, which followed the progressive wasting, cannot solely account for the cachexia. Bertini et al. (33) have reported that adrenalectomized mice were highly susceptible to IL-1 and TNF compared with normal mice. Hypercorticism may help to maintain the physiological homeostasis that had been perturbed by a large tumor burden.

Hematological analysis of mice bearing colon 26 revealed

Table 3 Changes in hepatic enzyme activities and the length of sleep induced by pentobarbital in mice bearing colon 26

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mice</th>
<th>Day 21</th>
<th>Day 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep (min)</td>
<td>Non-tumor-bearing 22 (12–29)</td>
<td>27 (15–41)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tumor-bearing &gt;1440D</td>
<td>&gt;1440D</td>
<td></td>
</tr>
<tr>
<td>P-450 (nmol/mg protein)</td>
<td>Non-tumor-bearing 0.25 ± 0.02</td>
<td>0.32 ± 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tumor-bearing 0.03 ± 0.04D</td>
<td>0.08 ± 0.03D</td>
<td></td>
</tr>
<tr>
<td>Catalase (K/g liver)</td>
<td>Non-tumor-bearing 9.63 ± 6.92</td>
<td>9.65 ± 4.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tumor-bearing 6.26 ± 2.14</td>
<td>2.97 ± 1.49D</td>
<td></td>
</tr>
</tbody>
</table>

* Median, with range in parentheses.

Table 4 Changes in plasma levels of fibrinogen, sialic acid, and IAP in mice bearing colon 26

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>Day 14</th>
<th>Day 21</th>
<th>Day 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>Non-tumor-bearing 148 ± 4D</td>
<td>127 ± 25</td>
<td>126 ± 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tumor-bearing 559 ± 112</td>
<td>818 ± 198</td>
<td>767 ± 169</td>
<td></td>
</tr>
<tr>
<td>Sialic acid (mg/dl)</td>
<td>Non-tumor-bearing 89 ± 1</td>
<td>82 ± 4</td>
<td>84 ± 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tumor-bearing 150 ± 4</td>
<td>158 ± 27</td>
<td>161 ± 10</td>
<td></td>
</tr>
<tr>
<td>IAP (µg/ml)</td>
<td>Non-tumor-bearing 156 ± 1</td>
<td>138 ± 21</td>
<td>111 ± 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tumor-bearing 1193 ± 123</td>
<td>1030 ± 211</td>
<td>1221 ± 179</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SD.
that extensive leukocytosis and slight anemia occurred as the tumor size increased. Three weeks after the tumor inoculation, when the maximum weight loss was attained, there were 3.6 times more leukocytes in the tumor bearers than that in normal mice. The number of leukocytes continued to increase until there were 11 times more of them on day 36. This leukocytosis was attributable to granulocytosis and was also observed in the noncachectic Meth A model. Since no bacterial or fungal infection was detected, the granulocytosis was possibly associated with tumor growth. IL-1 and TNF, known to cause granulocytosis (34) as well as cachexia (35, 36) in animal models, might act as possible mediators and cause the granulocytosis.

IL-1, TNF, and interferons are known to depress major drug-metabolizing systems, such as the microsomal P-450 enzyme systems, in the liver (37–39), and IFN is known to prolong pentobarbital-induced sleep in mice (39). Although the mechanism causing the depression of P-450 enzymes by IL-1 and TNF has not been clarified as of yet, it does accompany the acute phase response (40). TNF and IL-1 have been reported to stimulate the synthesis of certain proteins, which are markers of acute phase responses in the liver (41–43). We observed in the colon 26 cachectic mice that hepatic enzymes metabolizing drugs were suppressed and that plasma concentrations of the markers of acute phase responses (fibrinogen, IAP, and sialic acid-containing glycoproteins) were elevated. Furthermore, in drugs were suppressed and that plasma concentrations of the colon 26 cachectic mice that hepatic enzymes metabolizing systems, in the liver (37–39), and IFN is known to prolong metabolism in the cachectic tumor-bearing mice. These results indicate that the depression of drug-metabolizing enzymes requiring P-450-dependent metabolic activation, such as cyclophosphamide, may be different from that in non-cachectic tumor-bearing mice.

Experimental drug therapies have generally been carried out with mice that have just been implanted with tumor cell suspension, in which physiological conditions are quite different from those in mice with large tumor burdens. Such tumor models are also not indicative of the condition of cancer patients with cachexia or disorders of homeostasis. The present study shows that mice bearing colon 26 are truly cachectic, since we could identify some changes that were associated with cachexia. The cachetic colon 26 tumor-bearing mouse is an appropriate model for investigating mechanisms that cause cachexia and could be used as an alternative model for experimental drug therapies.

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